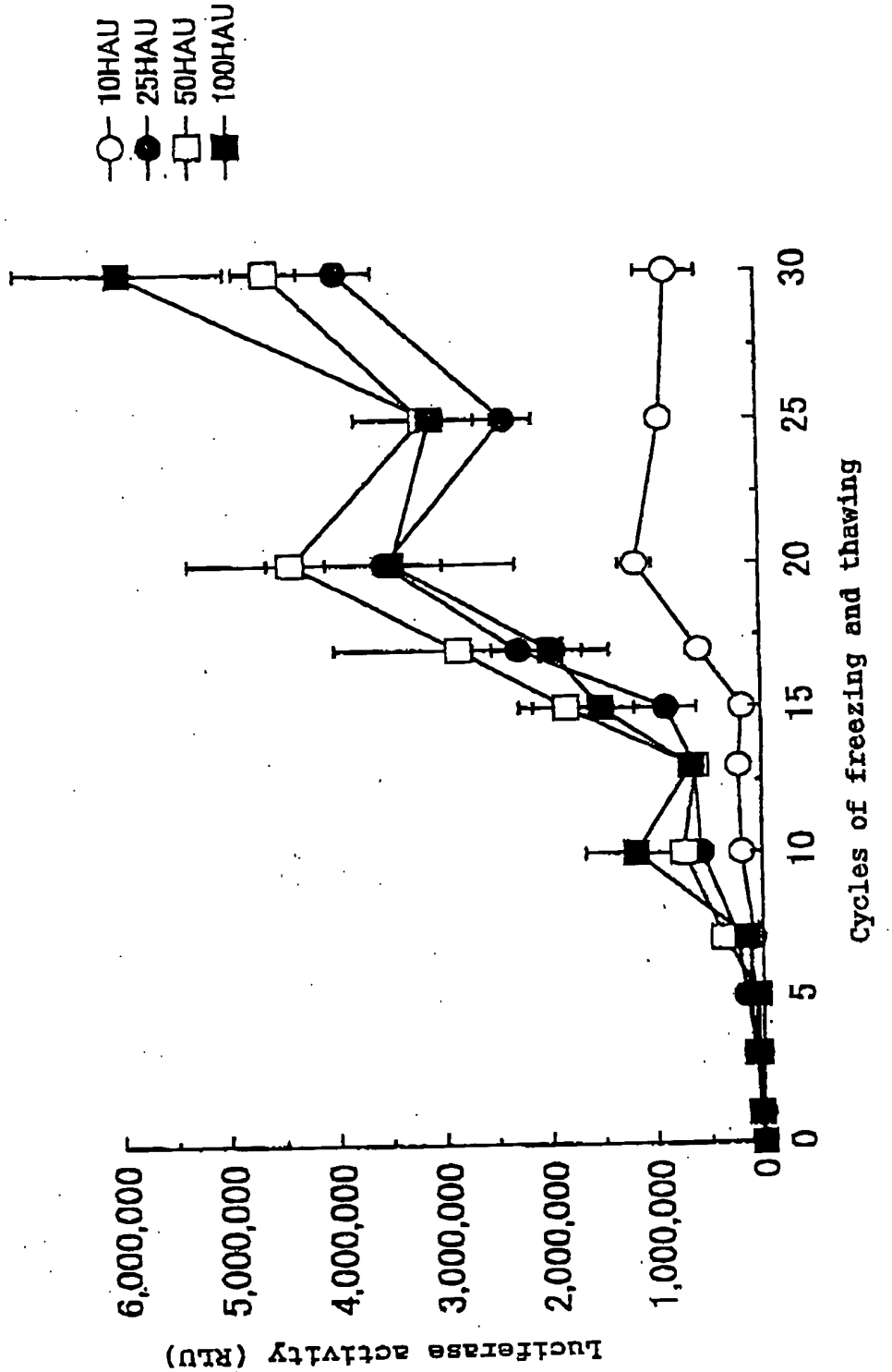

10HAU
25HAU
50HAU
100HAU
Luciferase activity (RLU)
6,000,000
5,000,000
4,000,000
3,000,000
2,000,000
1,000,000
0
0
5
10
15
20
25
30
Cycles of freezing and thawing

FIG. 1

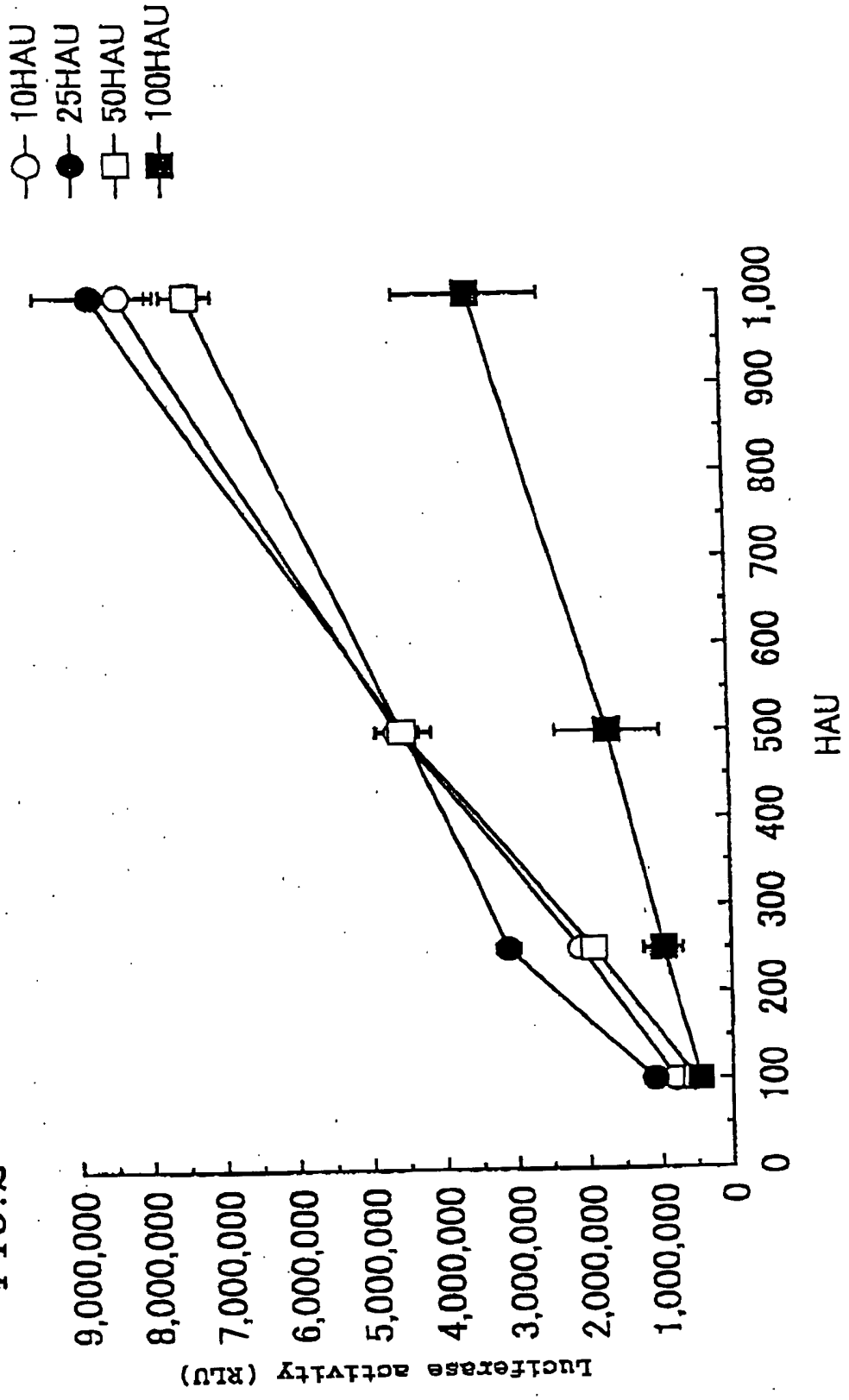

10HAU
25HAU
50HAU
100HAU
Luciferase activity (RLU)
9,000,000
8,000,000
7,000,000
6,000,000
5,000,000
4,000,000
3,000,000
2,000,000
1,000,000
0
100
200
300
400
500
600
700
800
900
1,000
HAU

FIG.2

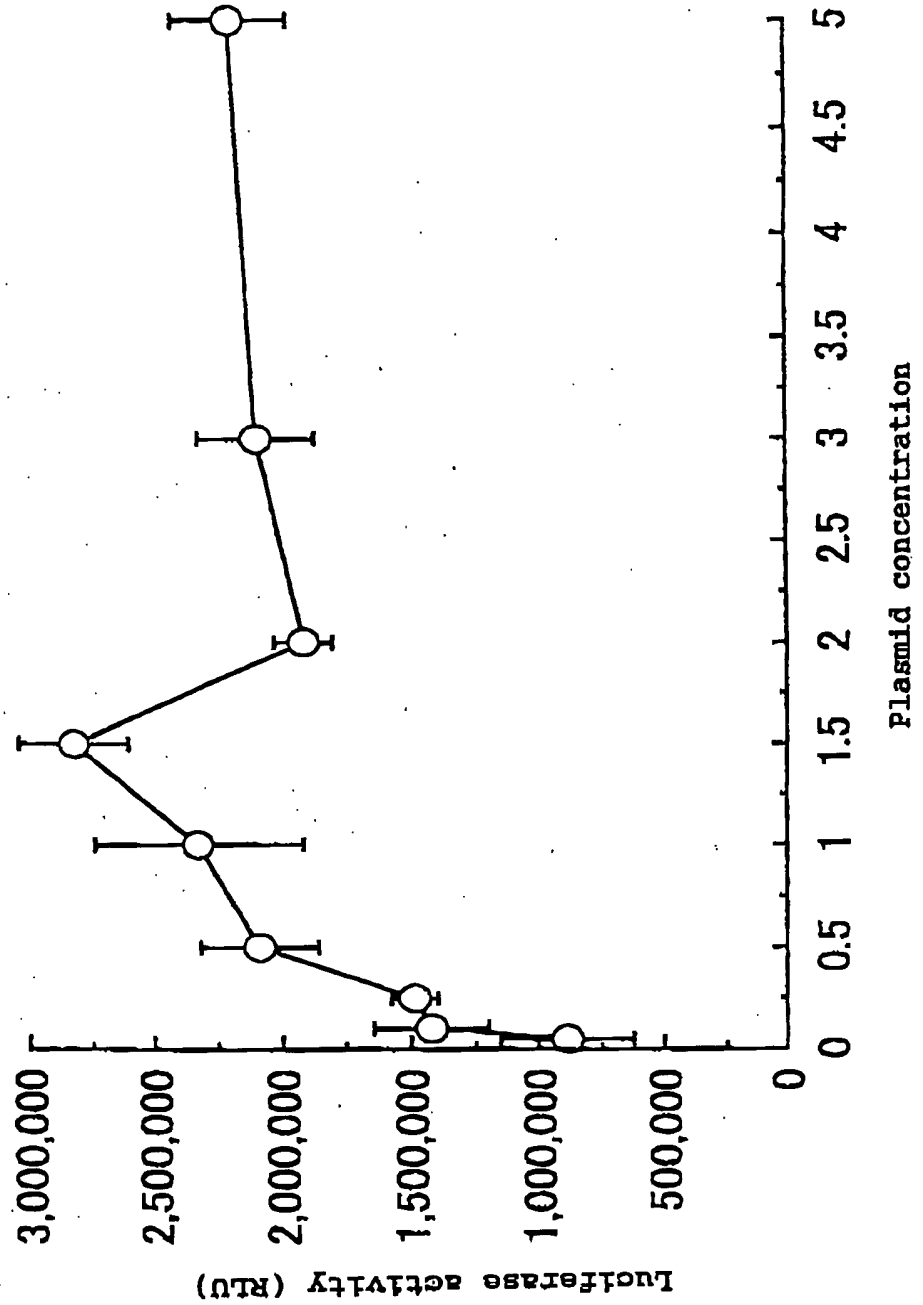

Luciferase activity (RLU)
3,000,000
2,500,000
2,000,000
1,500,000
1,000,000
500,000
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
Plasmid concentration

FIG. 3

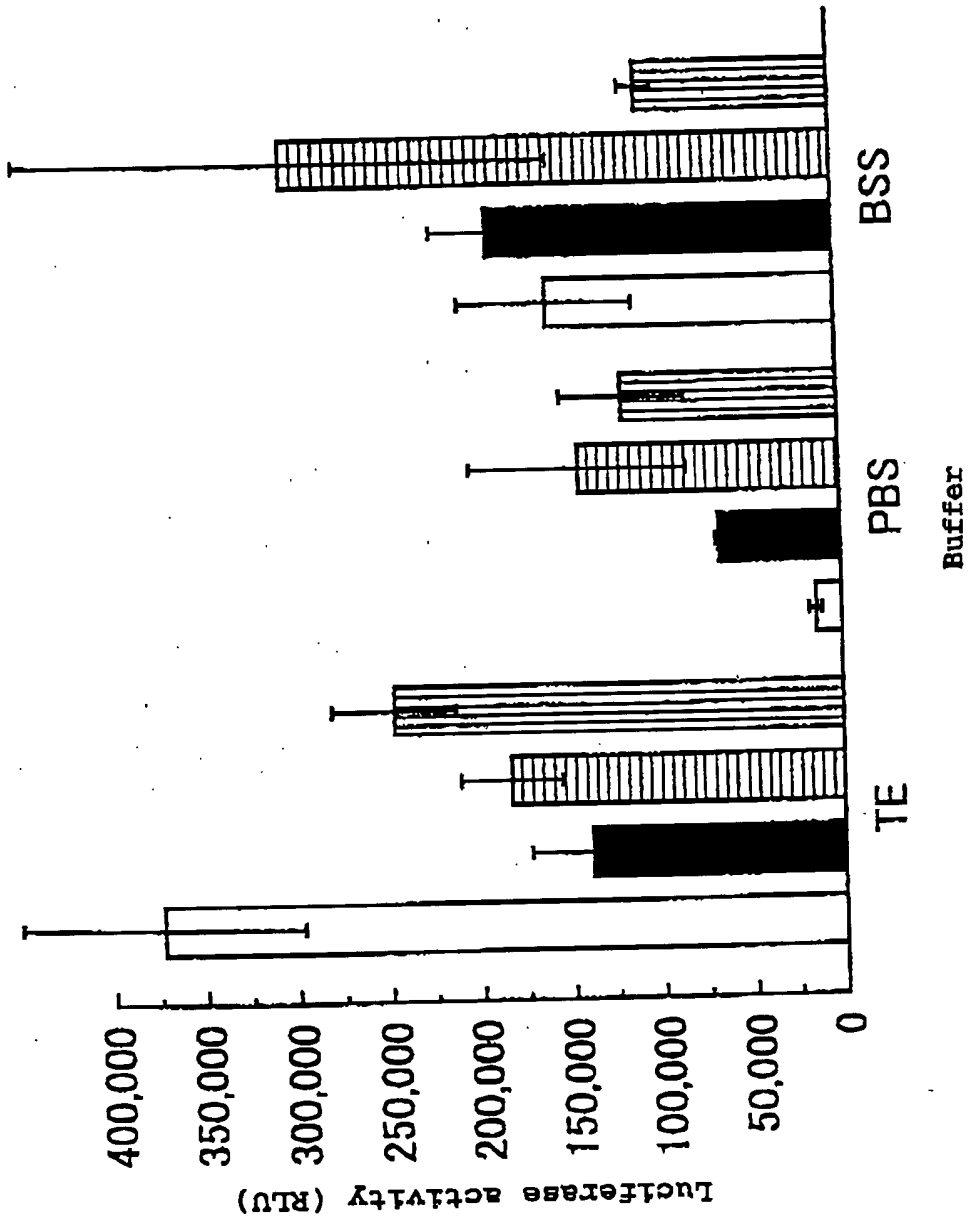

Buffer
20mM Sucrose
40mM Sucrose
60mM Sucrose
Luciferase activity (RLU)
400,000
350,000
300,000
250,000
200,000
150,000
100,000
50,000
0
TE
PBS
BSS
Buffer

FIG. 4

FIG. 5

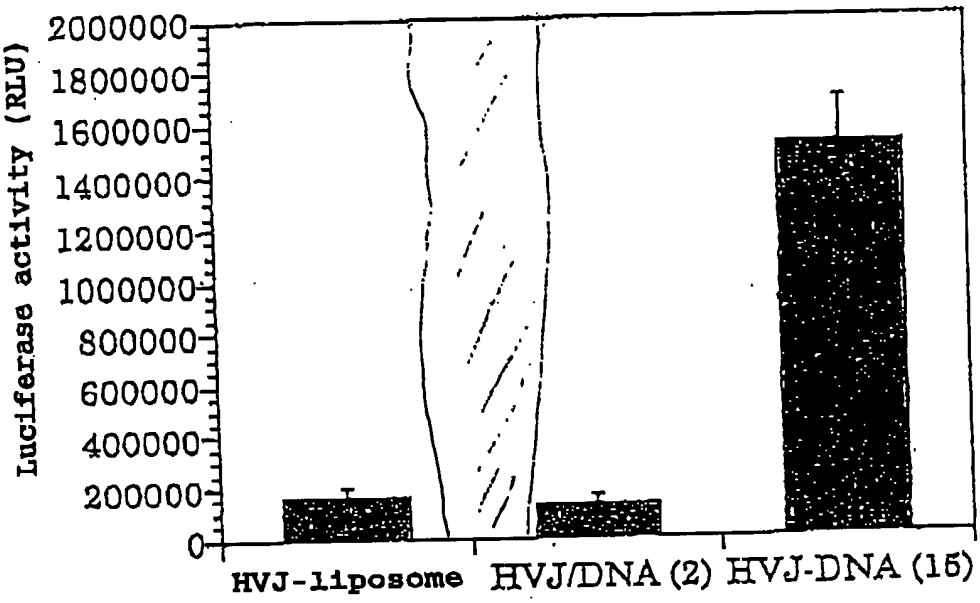


FIG. 6

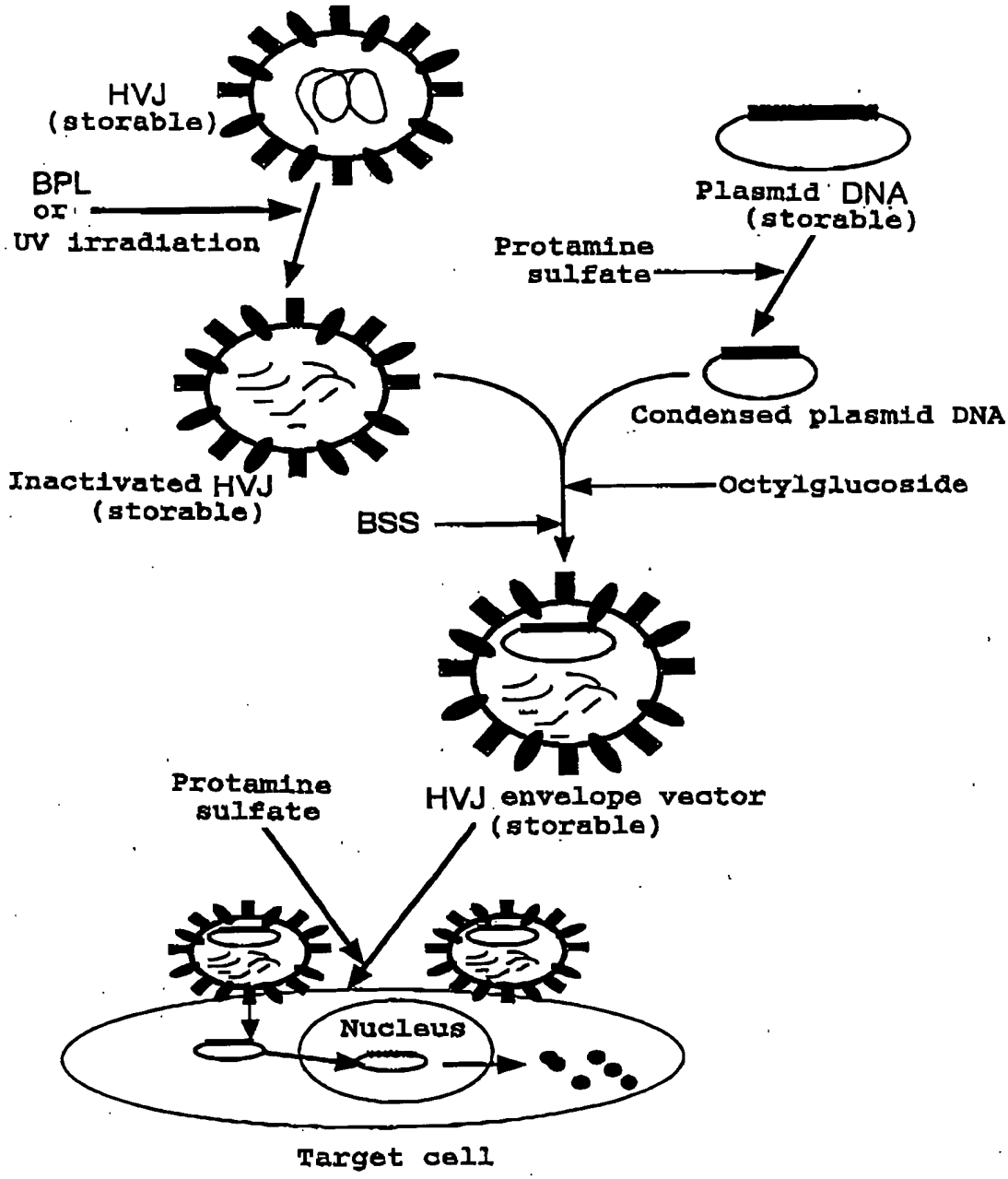

Preparation of HVJ envelope vector
HVJ (storable)
BPL or UV irradiation
Plasmid DNA (storable)
Protamine sulfate
Condensed plasmid DNA
Inactivated HVJ (storable)
Octylglucoside
BSS
Protamine sulfate
HVJ envelope vector (storable)
Nucleus
Target cell

FIG. 7

Protein profiles of HVJ, UV-inactivated HVJ, and HVJ envelope vector

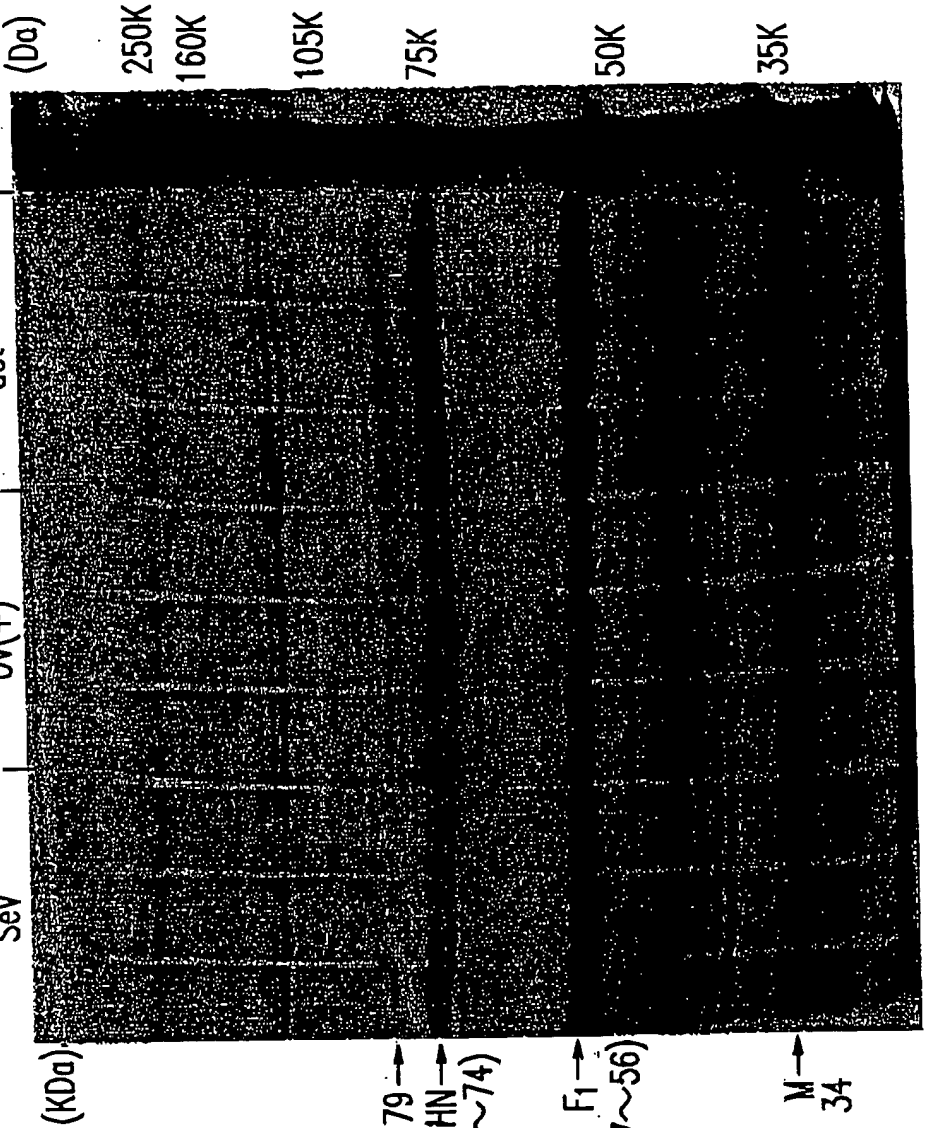
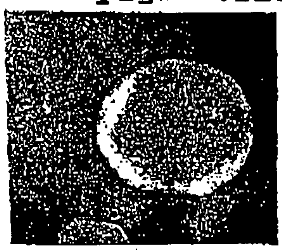


FIG.8

Electron micrograph of an HVJ envelope vector

(1) Untreated HVJ

(2) HVJ containing no DNA, which was subjected to an octylglucoside treatment



(3) HVJ containing DNA, which was subjected to an octylglucoside treatment

FIG. 9A

Effects of octylglucoside on gene transfer by HVJ envelope vector

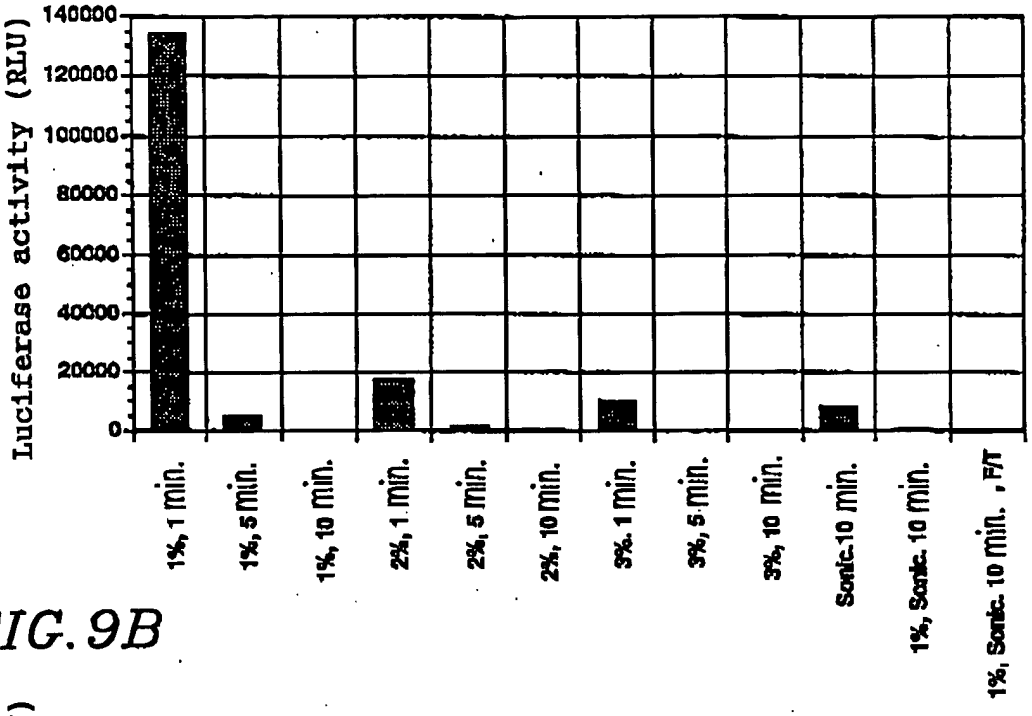


FIG. 9B

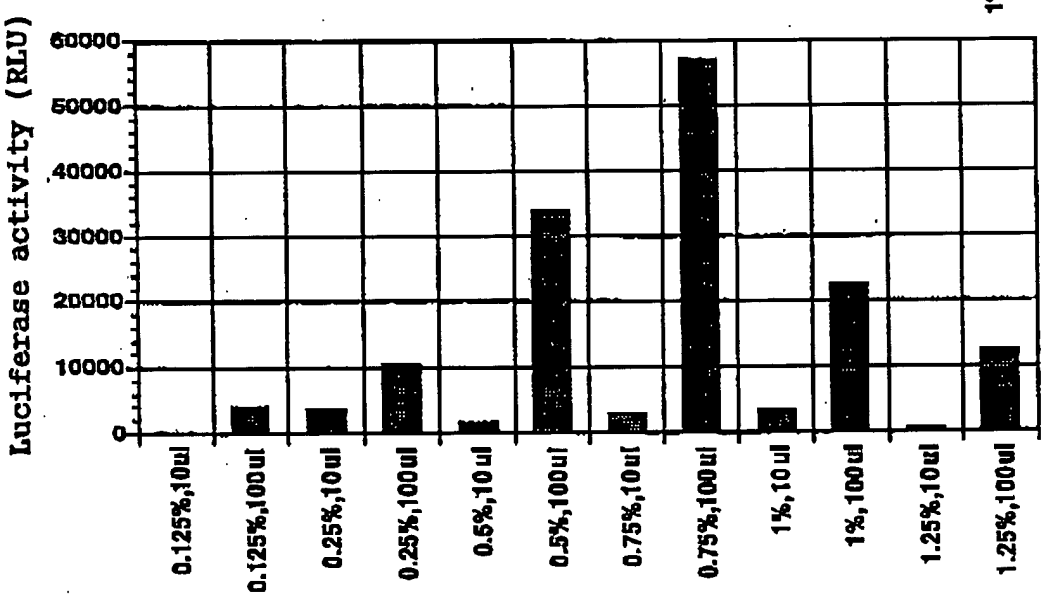


FIG. 9C

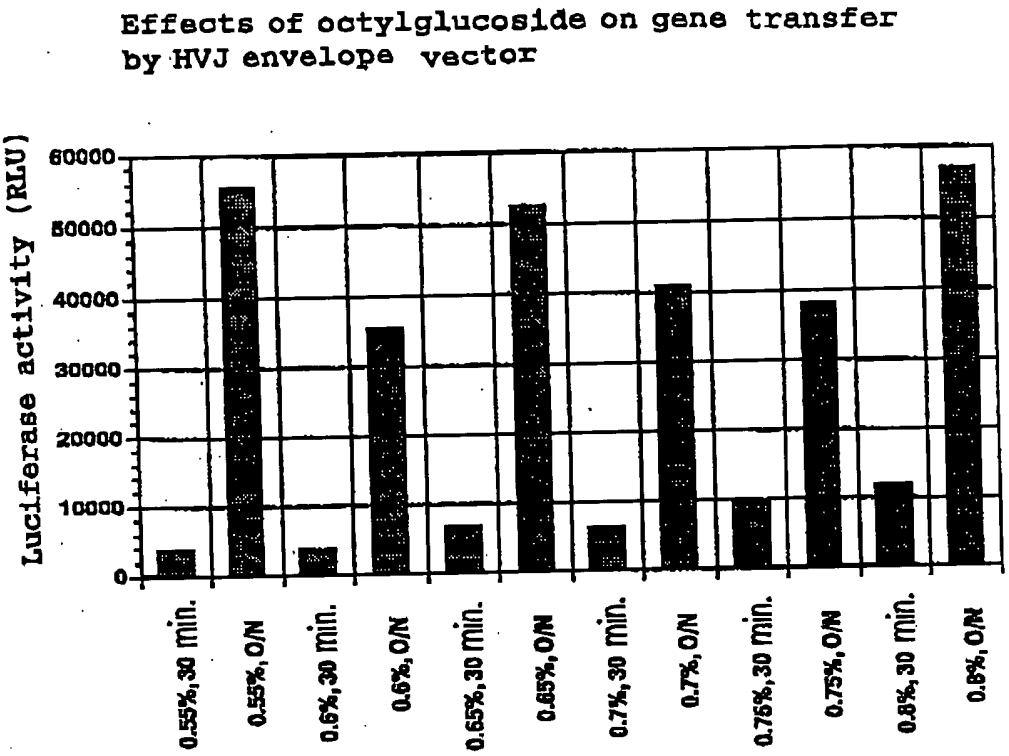

Effects of octylglucoside on gene transfer by HVJ envelope vector
Luciferase activity (RLU)
60000
50000
40000
30000
20000
10000
0
0.55%,30 min.
0.55%, O/N
0.6%,30 min.
0.6%, O/N
0.65%,30 min.
0.65%,O/N
0.7%,30 min.
0.7%, O/N
0.75%,30 min.
0.75%,O/N
0.8%,30 min.
0.8%, O/N

FIG. 10A

Effects of protamine sulfate on gene transfer by HVJ envelope vector

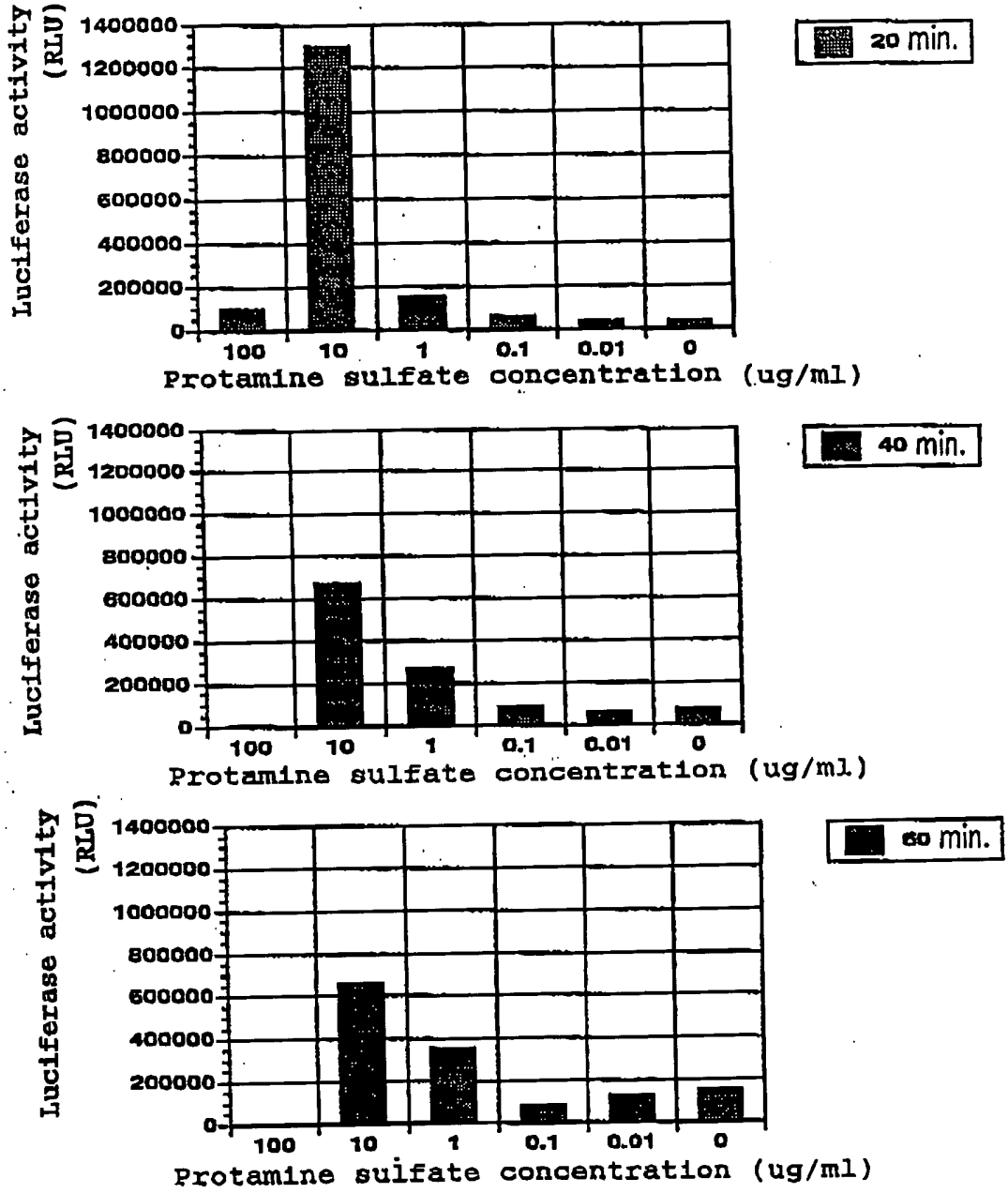


FIG. 10B

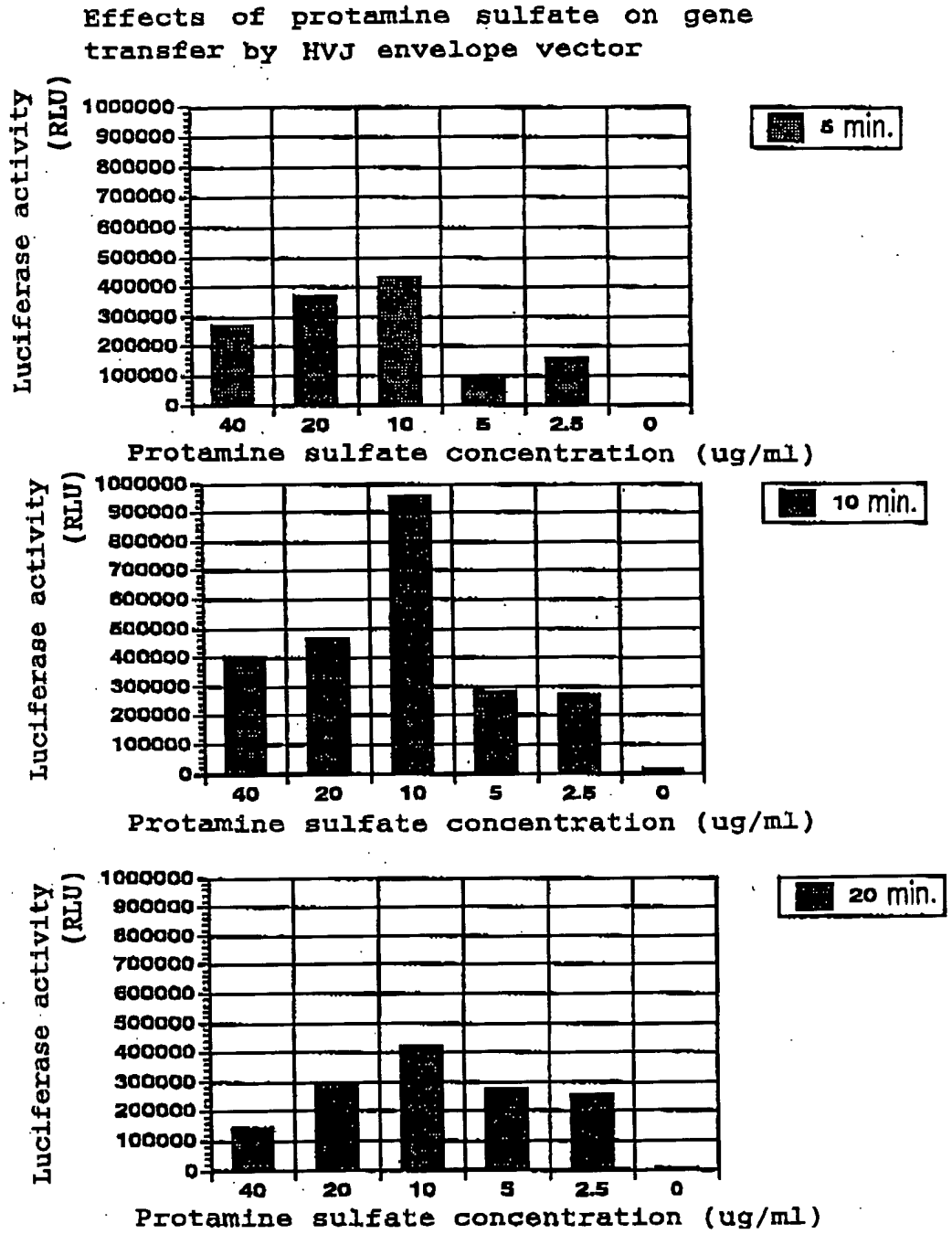
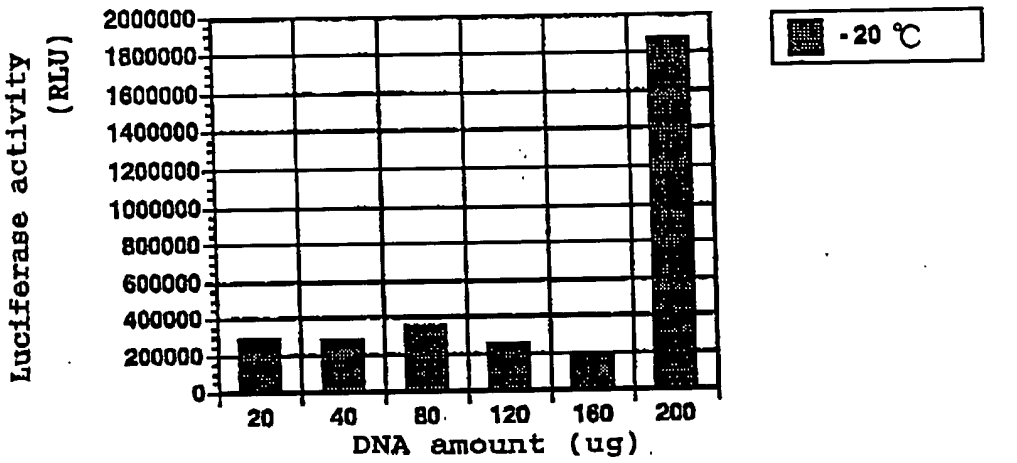

Effects of protamine sulfate on gene transfer by HVJ envelope vector
Luciferase activity (RLU)
1000000
900000
800000
700000
600000
500000
400000
300000
200000
100000
0
40
20
10
5
2.5
0
Protamine sulfate concentration (ug/ml)
5 min.
10 min.
20 min.

FIG. 11A

Effects of DNA amounts on gene expression using frozen HVJ envelope which has been treated with octylglucoside



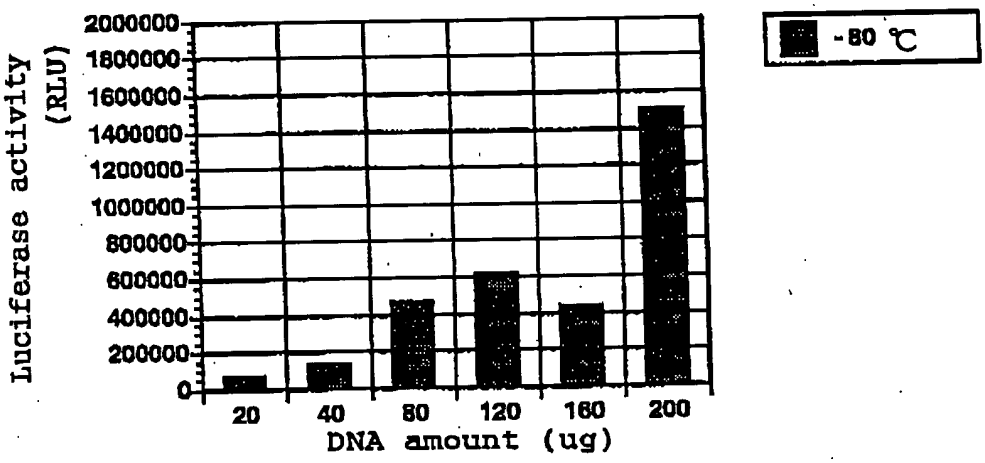


FIG. 11B

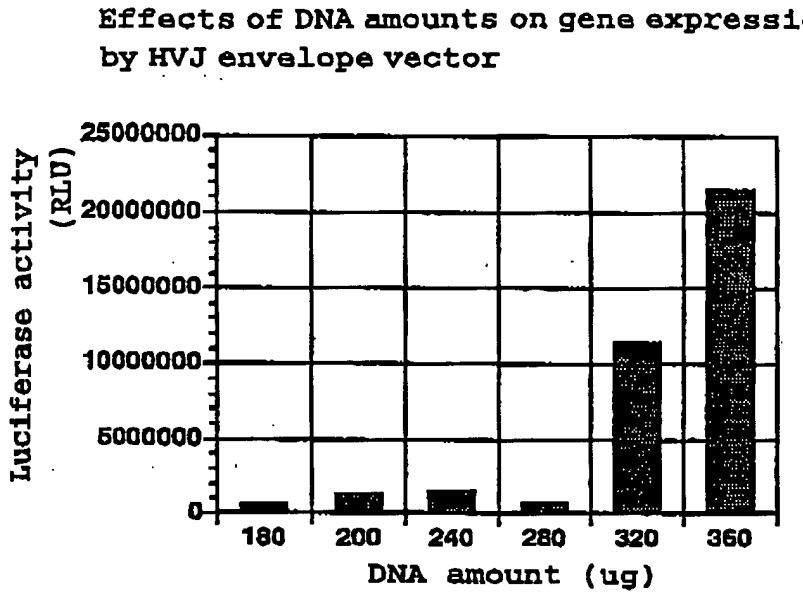


FIG. 12

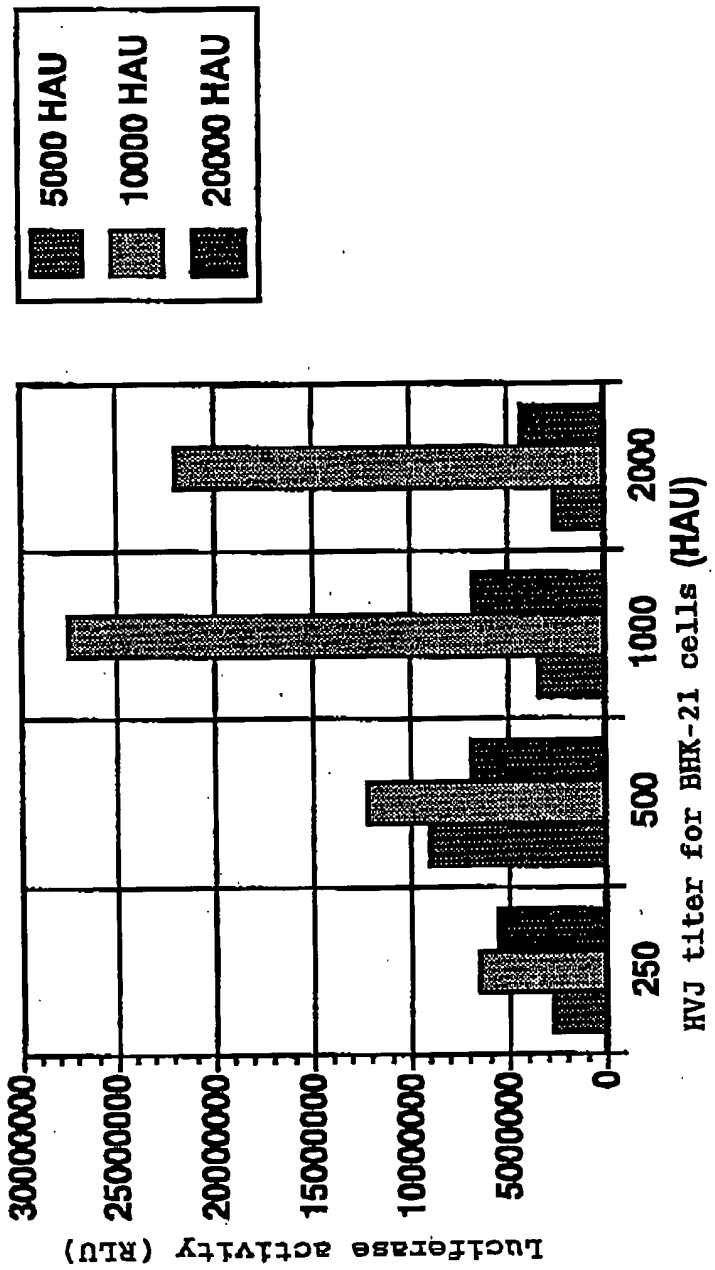

Effects of HVJ titer on gene expression
5000 HAU
10000 HAU
20000 HAU
Luciferase activity (RLU)
30000000
25000000
20000000
15000000
10000000
5000000
0
250
500
1000
2000
HVJ titer for BHK-21 cells (HAU)

FIG. 13A

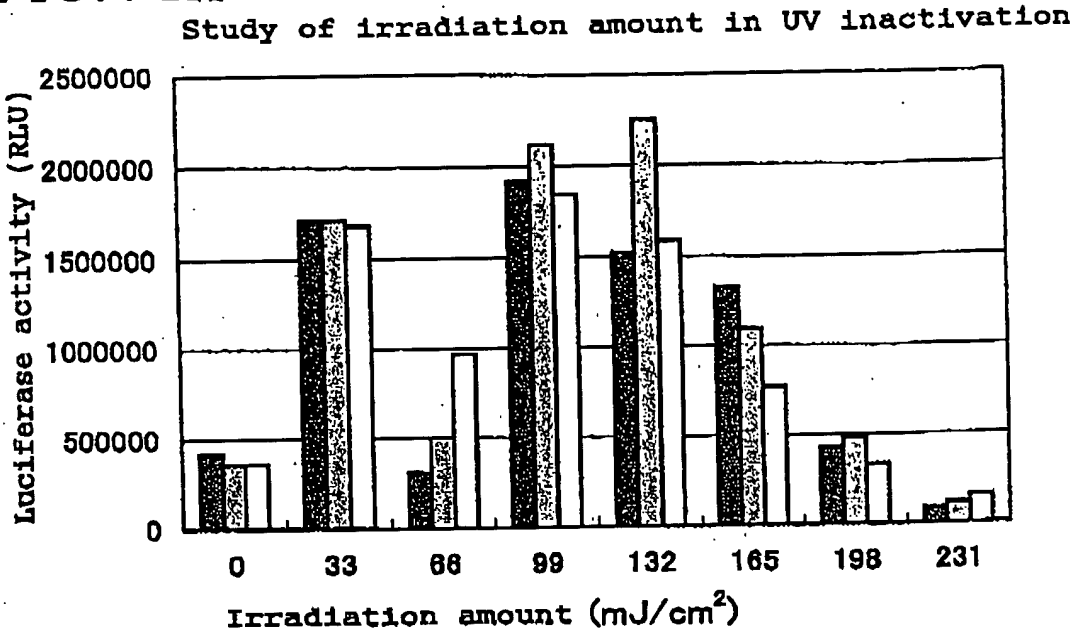


FIG. 13B

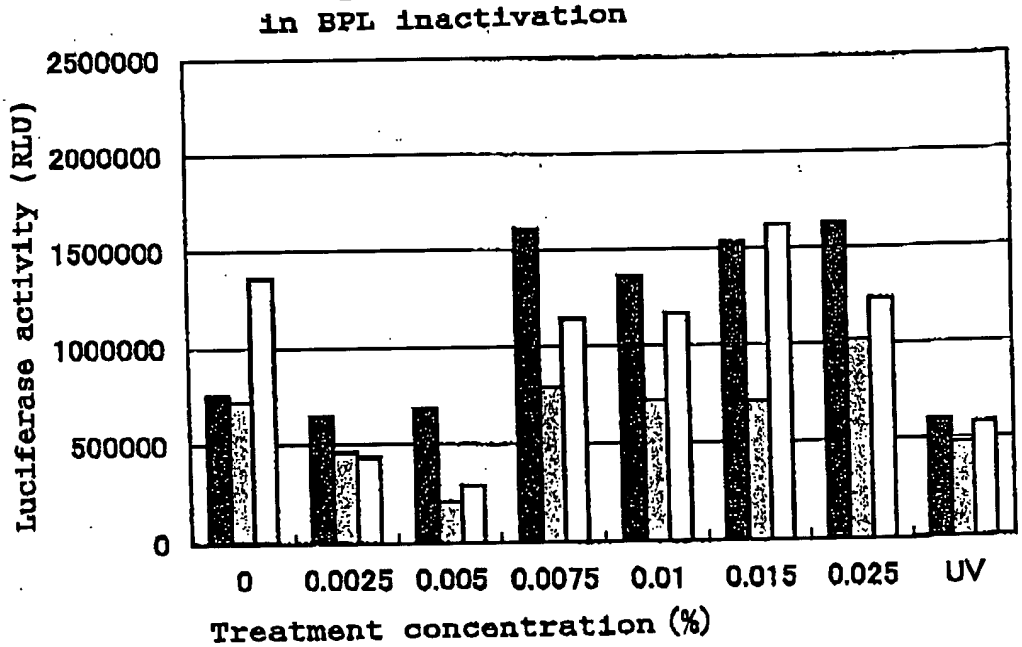


FIG. 14

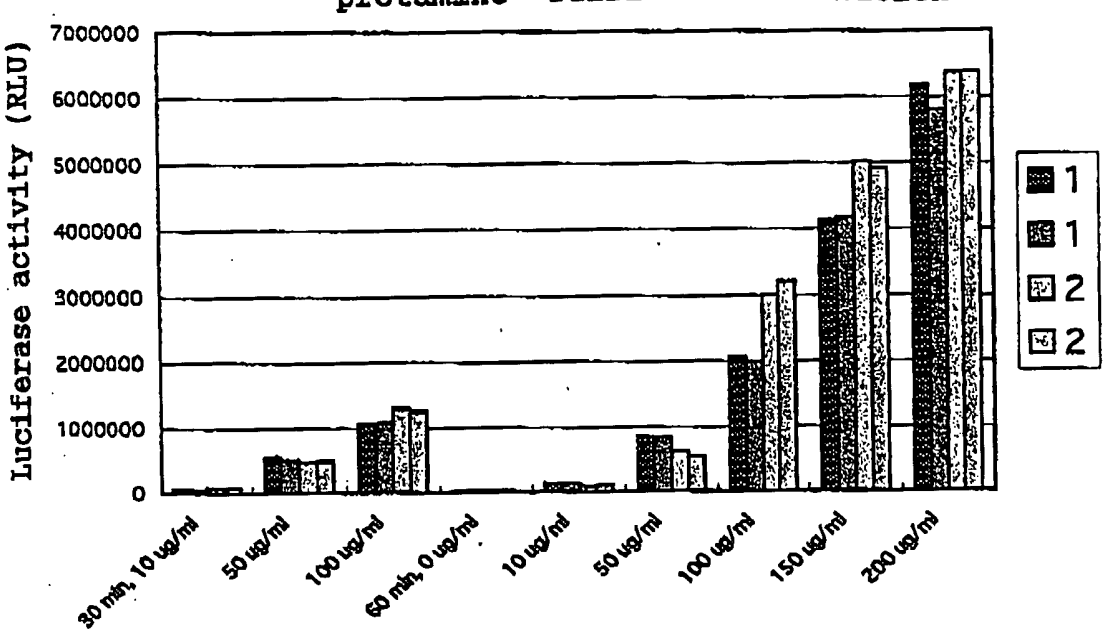

SAS: Effects of infection time and protamine sulfate concentration
Luciferase activity (RLU)
7000000
6000000
5000000
4000000
3000000
2000000
1000000
0
30 min, 10 ug/ml
50 ug/ml
100 ug/ml
60 min, 0 ug/ml
10 ug/ml
50 ug/ml
100 ug/ml
150 ug/ml
200 ug/ml
1
1
2
2
Infection time and protamine sulfate concentration

FIG. 15

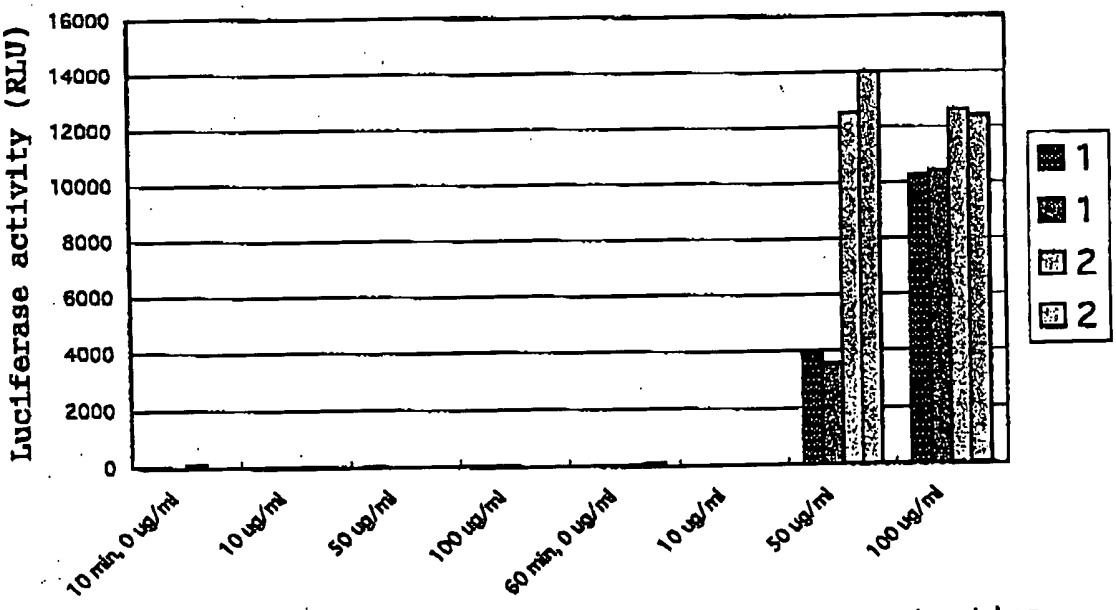

HAEC: Effects of infection time and protamine sulfate concentration
Luciferase activity (RLU)
16000
14000
12000
10000
8000
6000
4000
2000
0
10 min, 0 ug/ml
10 ug/ml
50 ug/ml
100 ug/ml
60 min, 0 ug/ml
10 ug/ml
50 ug/ml
100 ug/ml
1
1
2
2
Infection time and protamine sulfate concentration

FIG. 16A

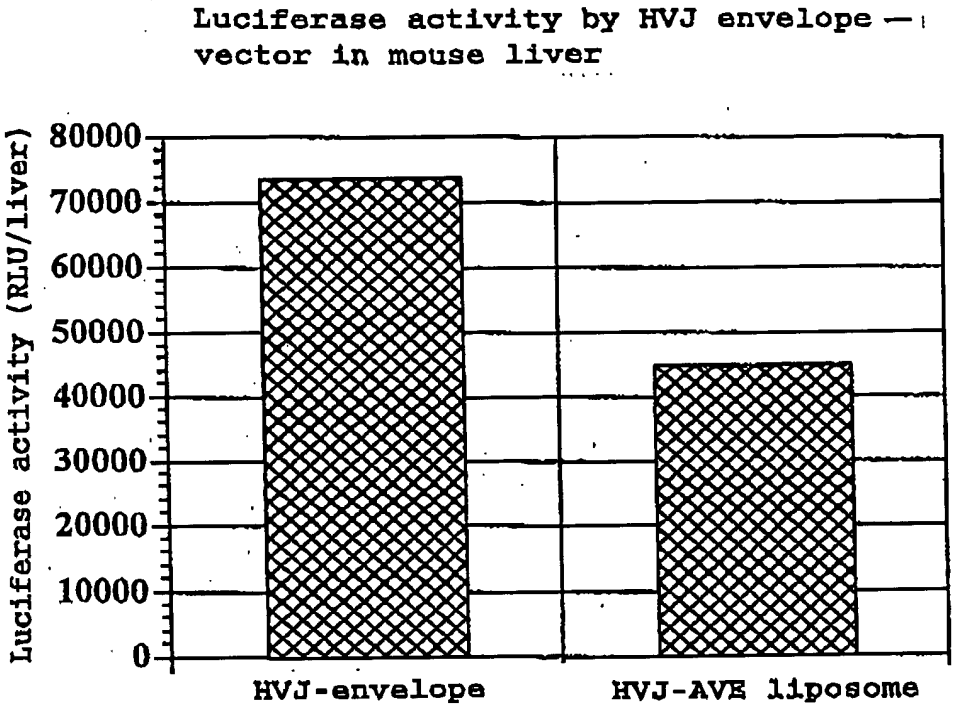

Luciferase activity by HVJ envelope — vector in mouse liver
Luciferase activity (RLU/liver)
80000
70000
60000
50000
40000
30000
20000
10000
0
HVJ-envelope
HVJ-AVE liposome

FIG. 16B

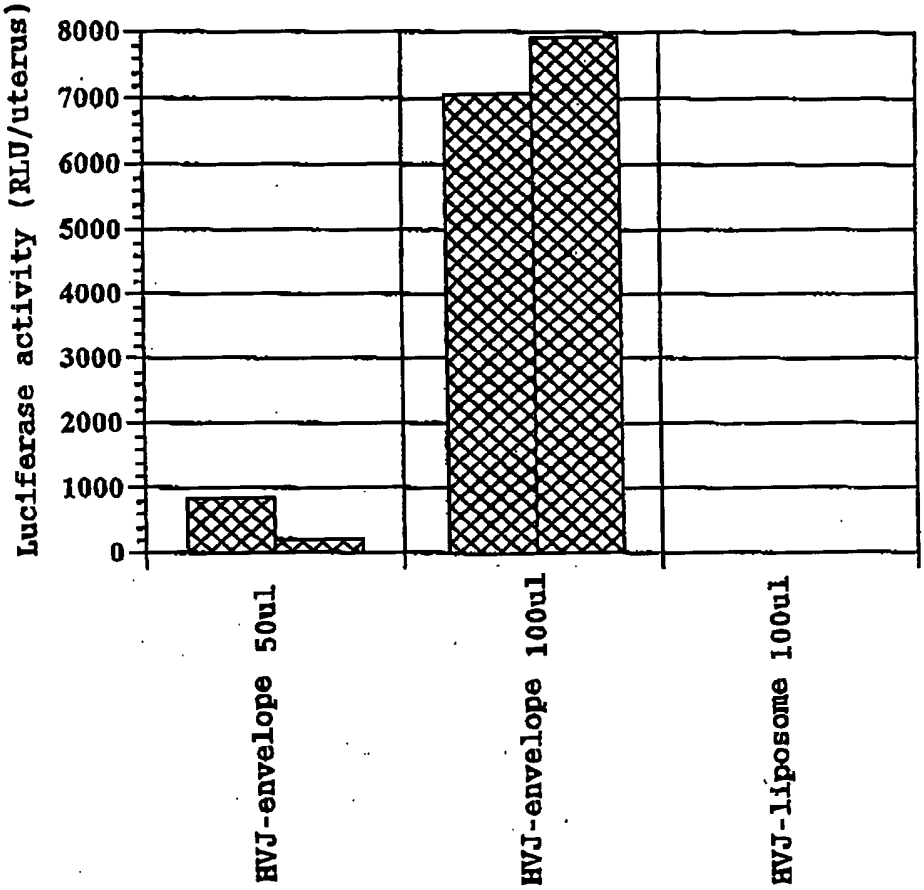

Luciferase activity by HVJ envelope vector in mouse uterus
Luciferase activity (RLU/uterus)
8000
7000
6000
5000
4000
3000
2000
1000
0
HVJ-envelope 50ul
HVJ-envelope 100ul
HVJ-liposome 100ul

FIG. 16C

LacZ expression by HVJ envelope vector in mouse uterus

FIG. 16D Gene transfer into rat brain

Gene transfer into central nervous system using new HVJ

#1 HVJ-GFP

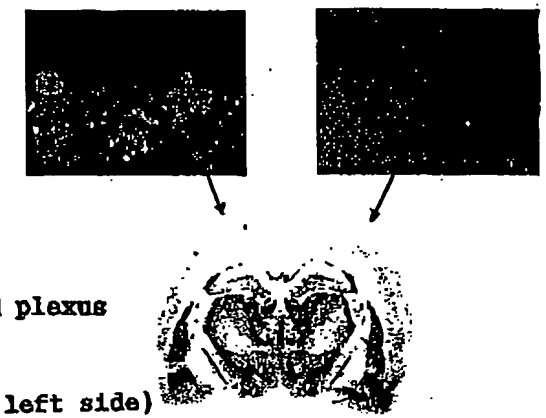
HVJ-GFP of 10,000 HAU was administered to SD rats (male, body weight: 300 to 400 g) via the cisterna magna or via the carotid artery. Samples were taken three to four days later.

Live sections were prepared, which were subjected to observation under fluorecence microscopy.

(administration via the cisterna magna) ①

Incorporation into the brain surface was confirmed.
No incorporation into deep portions of the brain was confirmed.
No incorporation into the chorioid plexus was confirmed, either. → administration via the cisterna magna is considered to result in permeation through the intrathecal space, so that expression is usually observed in the chorioid plexus

② Administration via the carotid artery



(administration via the carotid artery) ②, ③

Significant expression was confirmed on the administered side (left side) Expression was confirmed not only in the brain surface portions but also in the basal ganglia portion. Expression was also confirmed in the brain surface of the other brain, which was considered to have resulted from a flow to the other side through a colateral flow.

① Administration via the cisterna magna

③ Administration via the carotid artery

FIG. 16E **Inhibition of VEGF-induced angiogenesis by gene transfer using HVJ**

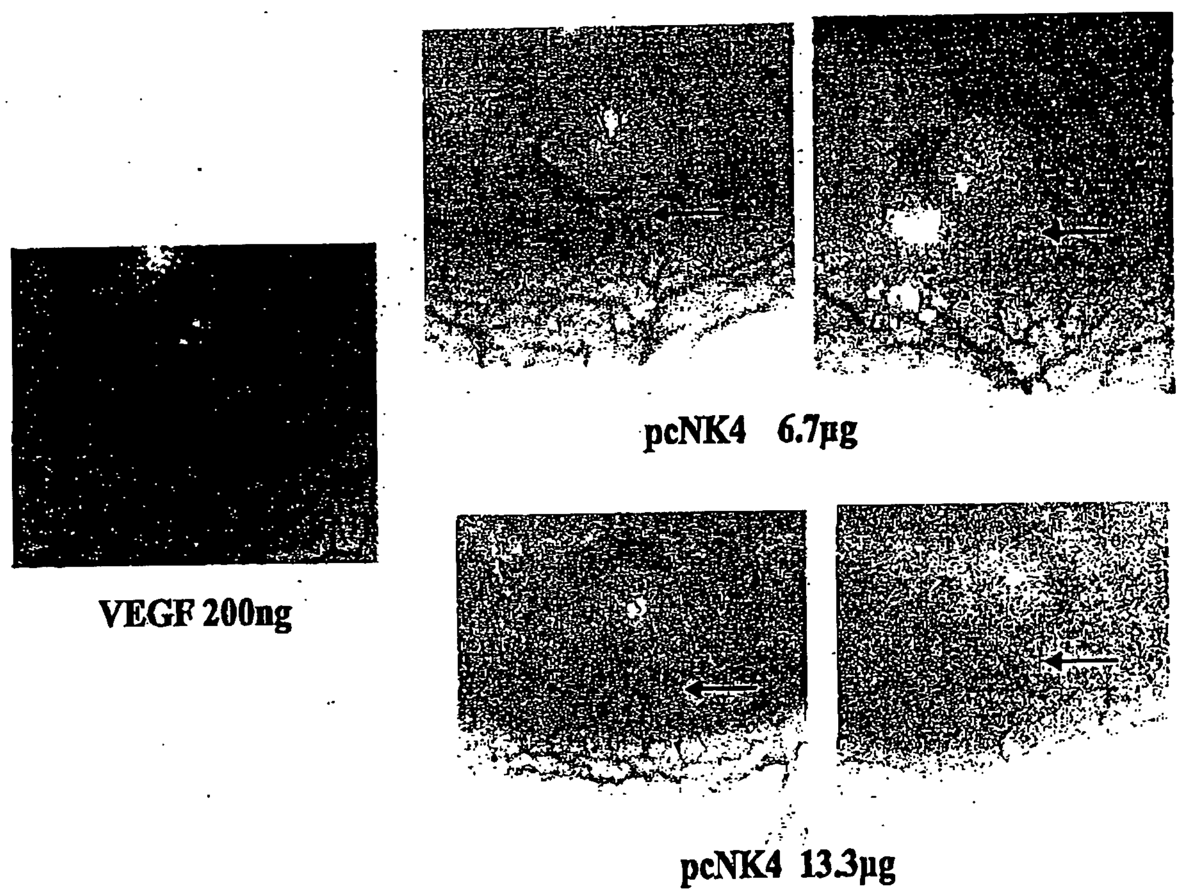


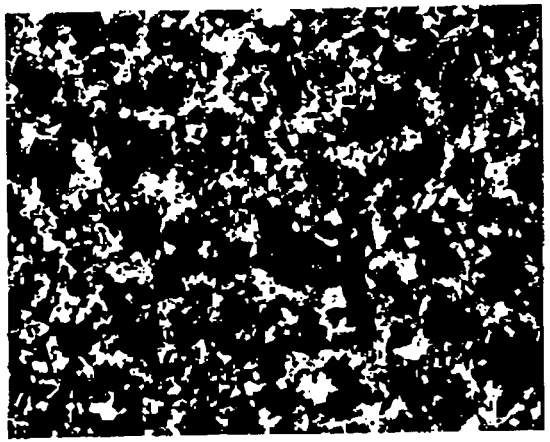
FIG. 16F

Introduction of FITC-ODN into BHK-21 cells by HVJ envelope vector

FIG. 17A

60 min

Fluorescence image



Phase-contrast image

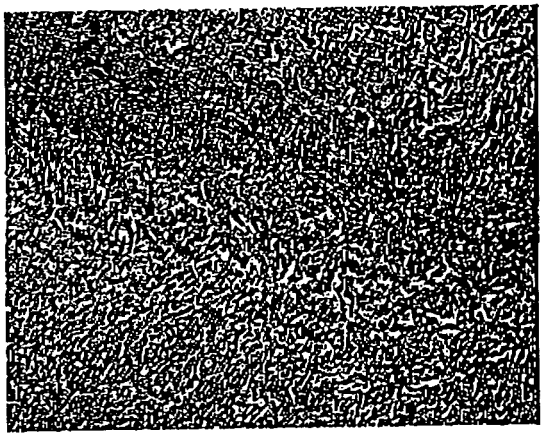


FIG. 17B

10 min

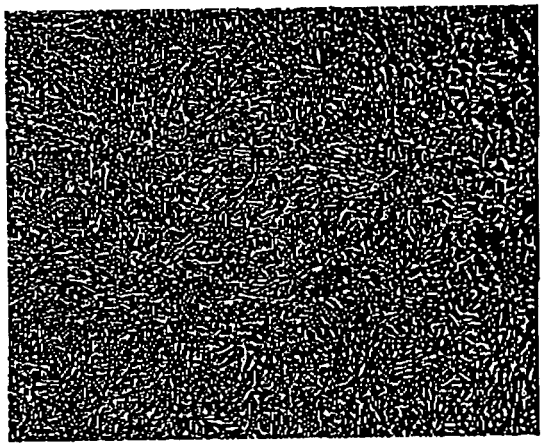


FIG. 18A

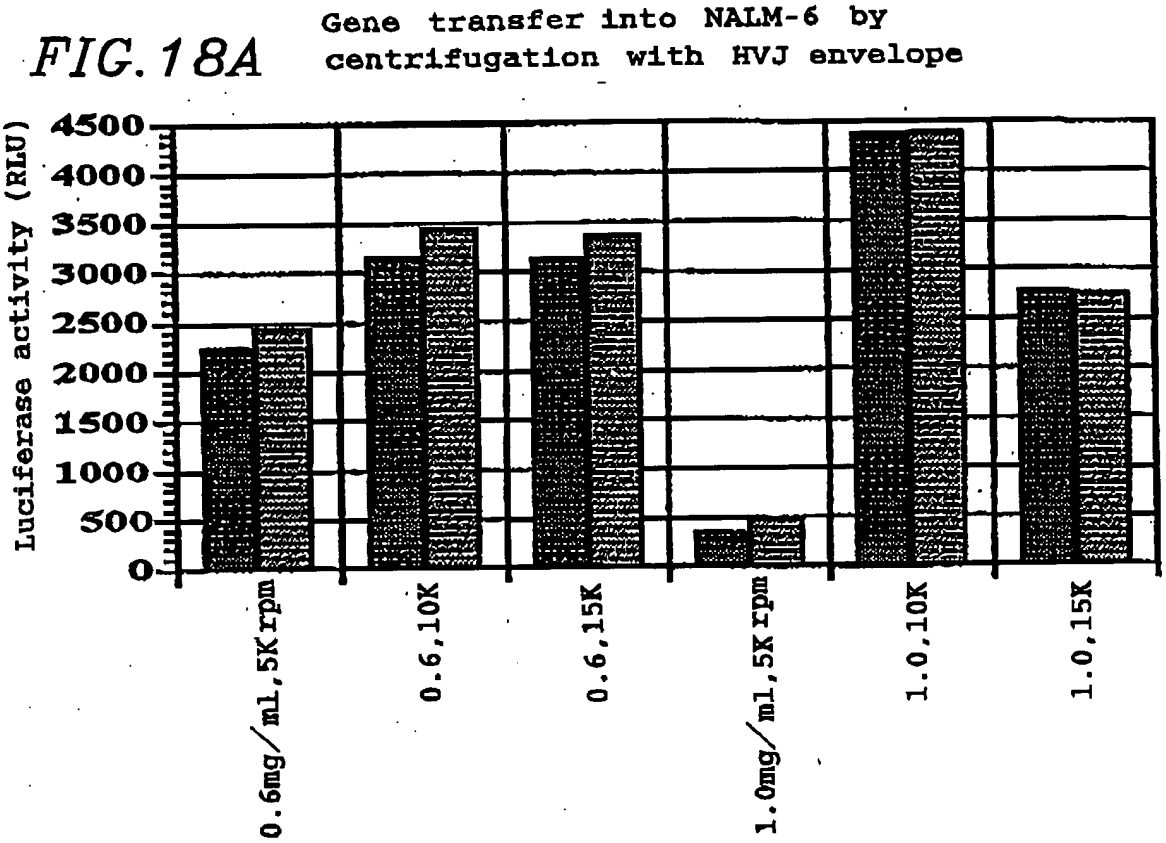

Gene transfer into NALM-6 by centrifugation with HVJ envelope
Luciferase activity (RLU)
4500
4000
3500
3000
2500
2000
1500
1000
500
0
0.6mg/ml,5Krpm
0.6,10K
0.6,15K
1.0mg/ml,5Krpm
1.0,10K
1.0,15K
Protamine sulfate concentration and centrifugation

FIG. 18B Gene transfer into CCRF-CEM by centrifugation with HVJ envelope

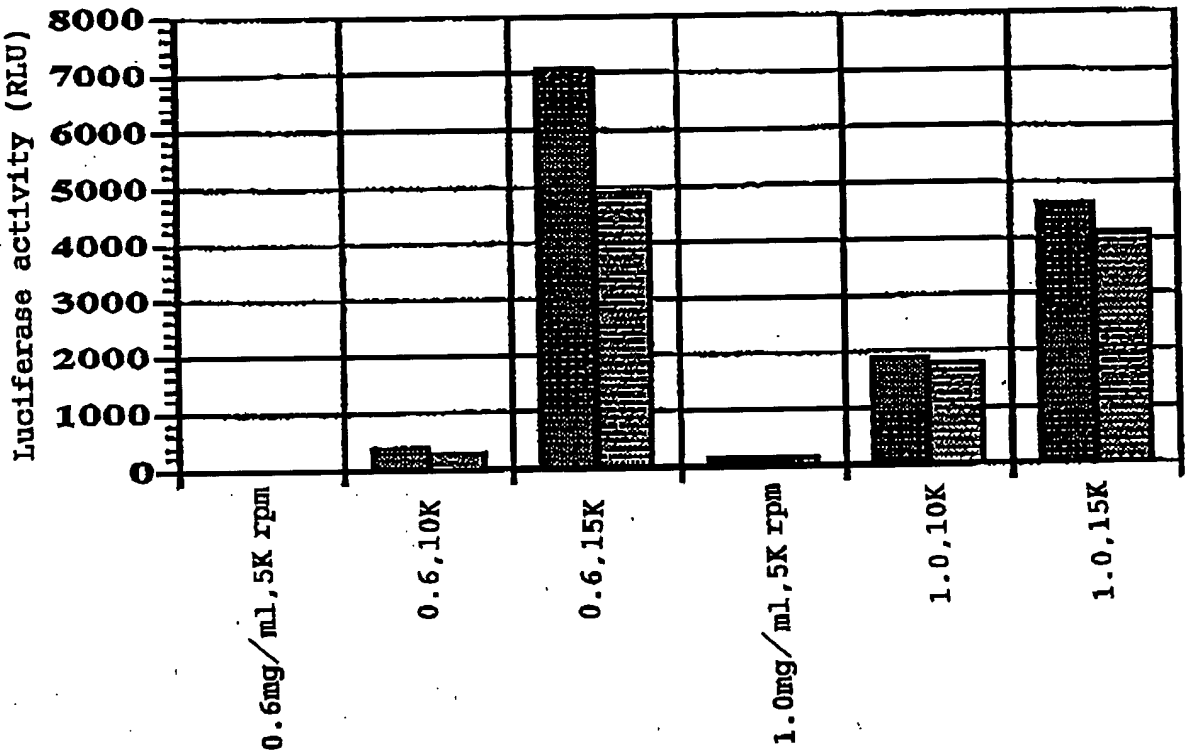


FIG. 18C

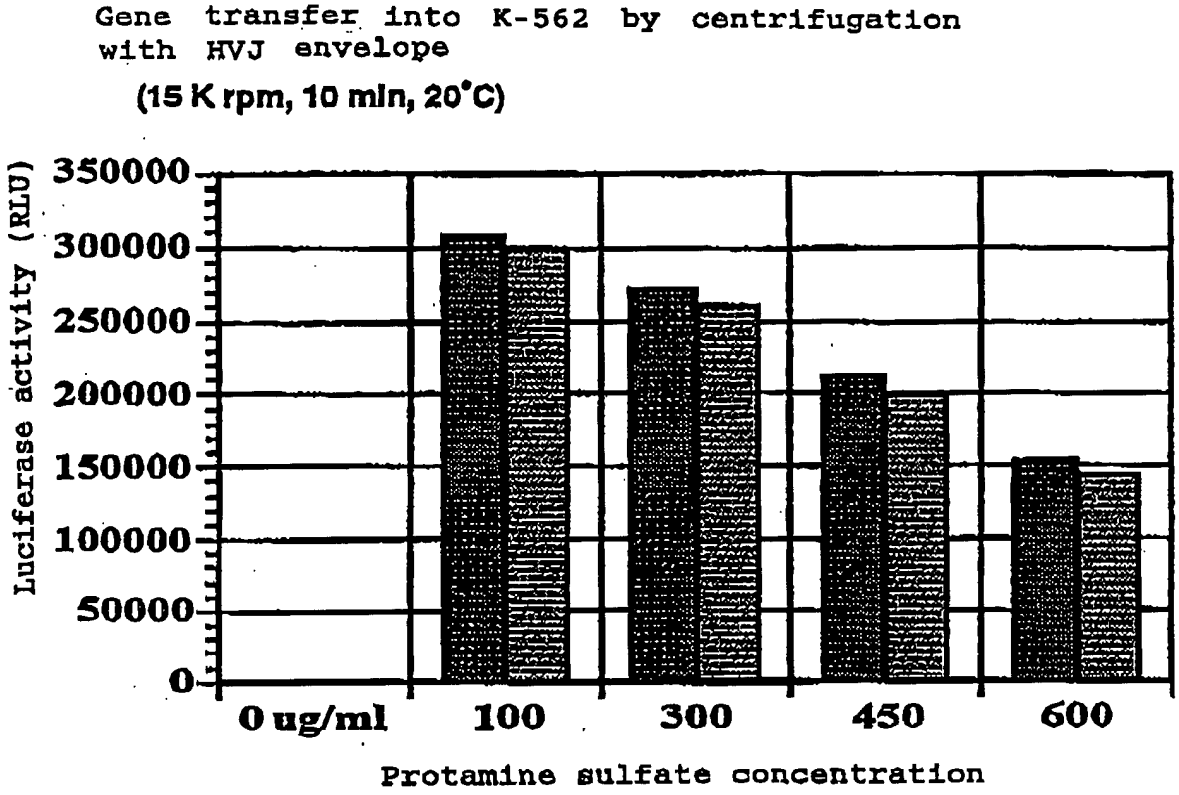


FIG. 19

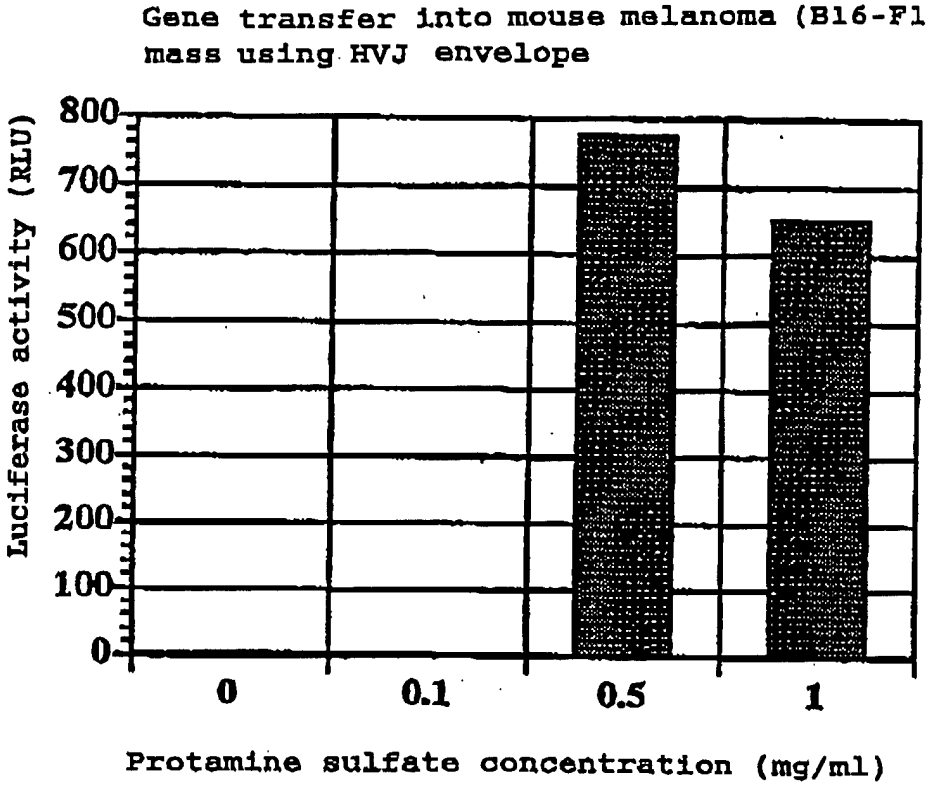


FIG.20

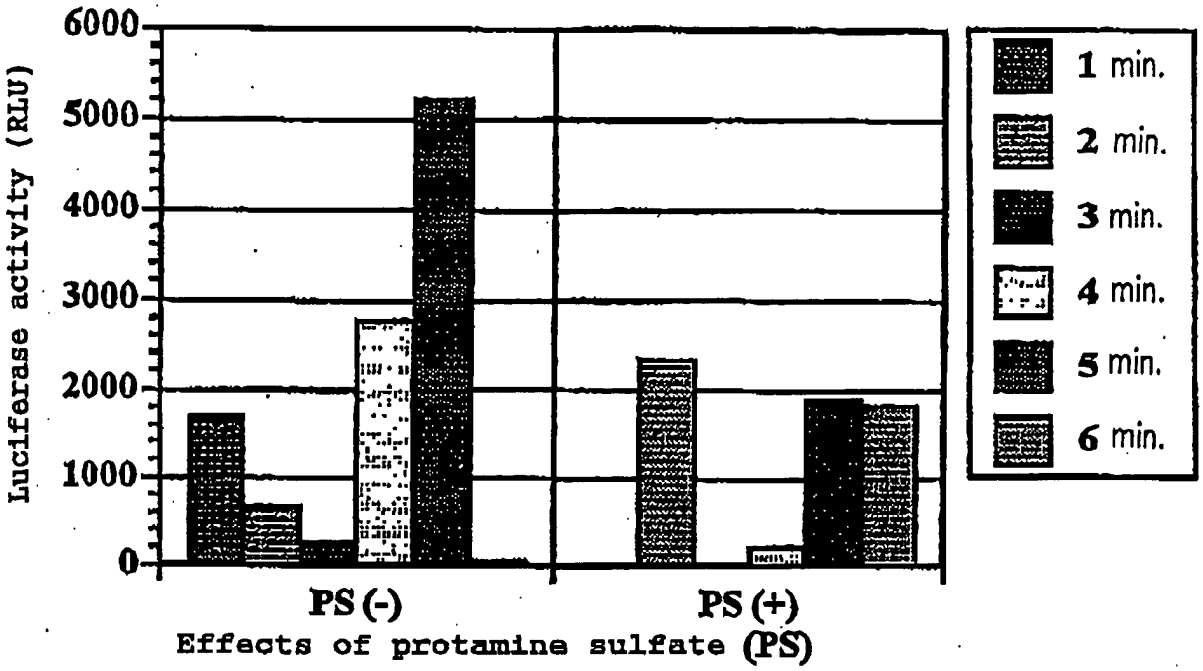


FIG.21